Quarterly Progress to:	National Institutes of Health
Contract Monitor:	William Heetderks, Ph.D.
Research Contract:	"Surface Modification for Biocompatibility"
Contract No.:	NS 5-2322 QPR#10
Principal Investigators:	David C. Martin and K. Sue O'Shea
Date:	July 31, 1997

Overview

This report is a summary of our activities in the tenth quarter of our contract, corresponding to the second quarter of 1997. In this tenth period of activities, we have continued developing and refining our processing characterization techniques. In addition, we have initiated an *in vivo* study of silicon probes coated with the protein polymers in the Guinea Pig CNS. This report provides an overview of the major results to date and discusses our plans for the future. We have been working to (1) characterize protein polymer films, (2) develop assays to evaluate bioactivity of protein polymer films *in vitro*, and (3) evaluate bioactivity and stability of protein polymer films *in vivo*. We also describe our efforts to discuss our work in (4) external communications with the scientific community.

1. Protein Film Deposition, Morphology, and Device Characterization

Progress:

We have continued to analyze the mechanical properties of the polymer coatings on silicon substrates. In last quarter's report we described the use of nanoindentation experiments to look at the modulus of protein polymer coatings of various morphologies on silicon. Figure 1 shows an SEM of an indented, filamentous film of SLPF. The densification of the film, which leads to an increase in stiffness, is evident in the region near the center of the conical indentation pattern left on the film surface. Figure 2 shows plots of load (in mN: 1 mN full scale, top figure, 10 mN full scale, bottom figure) as a function of displacement (in nm) for films of different thicknesses. By comparing the response of these curves as a function of load, it is possible to extract information about variations in modulus for SLPF films with different morphologies.

Figure 3 shows a plot of the resulting data, shown in the manner of the modulus as a function of distance. Also shown are estimates of the mechanical properties of silicon (172 Gpa, Weppelmann et al., 1993) and brain (100 kPa, Hirakawa et al., 1981) available from the literature.

All of the SLPF polymer coatings help to mediate the dramatic difference in mechanical properties, since the moduli are intermediate to that of the extremely stiff silicon and the soft tissue. However, the filamentous coating appears to be able to broaden out the mechanical properties gradients most effectively. It also seems likely that the "hairy" geometry of this coating will be more efficient in promoting efficient cellular attachment and adhesion than flatter continuous and beaded coatings.

We have also continued our analyses of the electrical performance of the probes by impedance spectroscopy (IS), and have refined our estimates of the characteristics of the charge transport process. Our data has facilitated the characterization of the surface

roughness of probe, and by comparing the regimes of power law behavior observed as a function of temporal frequency in these measurements with similar power law behavior observed in atomic force microscopy analyses of the surface morphology, we have been able to estimate diffusion coefficients of the transport process controlling carrier motion at the interface. The diffusion coefficient, which can be estimated as:$D = w/q^2$, can be determined from the limiting values for w and q. From the IS measurements, we find power law behavior below a frequency of 10^5 sec^{-1}, and above 10 sec^{-1} (estimated). From the AFM measurements, the spatial frequencies for power law behavior are above 0.5 um^{-1} and below 40 um^{-1} (estimated). Combining these two estimates gives values of 4-6 x 10^{-7} cm^2/sec for D. This value is consistent with the known diffusivities of cations in proteins (Brandup and Immergut, Polymer Handbook, 1966).

Plans:

We are continuing to analyze our mechanical properties data in terms of theoretical models of deformation as a function of morphology. Lately, we have found that by plotting the indentation load P as a function of displacement d on log-log coordinates we can determine the values of characteristic prefactor C and exponent n in the expression

$$P = C\,d^n$$

For continuous films, n is expected to vary between 1 and 2 depending on the geometry of the sample. We are now comparing our results to those obtained on other materials and will elaborate on these results in next quarter's report.

These results have also motivated us to consider new designs for protein polymer materials that specifically facilitate ionic transport.

2. Bioactivity of Protein Polymer Films *in vitro*

Progress:

The proposal to the National Nanofabrication Facility was accepted, and Dr. Libby Louie fabricated a series of designs of systematically varying (2-50 micron) dimensions (Figure 1'). Patterns were created to incorporate a 90 degree dogleg at the end of each channel to test substrate specificity. Coverslips were coated with photoresist, UV light irradiated through the patterned mask onto the photoresist. The UV reacted photoresist was washed away, leaving a pattern of unreacted photoresist and bare glass. Proteins were absorbed onto the glass surface, a secon wash removed the remaining photoresist, producing a pattern of protein and glass (Figure 2').

db-cAMP primed Neuro-2A cells were added to these patterned coverslips at a concentration of 1 x 105 cells/ml and allowed to attach for 1 hour at 37 C. Unattached cells were removed, and cultures grown for an additional 2-24 hours. Neurons recognized the patterned substrates and neurites and cell bodies were observed to bend around the turn of the dogleg (Figure 3'). Neuronal cells respect the patterned boundaries when the distance between lanes is greater than 10 microns, 2 micron spaces were breached by cell processes and eventually by cell bodies. In addition to parameters such as neurite length and branching, we are examining the ability of cells to signal via focal adhesions using antibodies to focal adhesion kinase and phosphotyrosine with laser confocal microscopy. We are comparing the ability of fibronectin, SLPF, laminin, and SLPL to support neuronal adhesion, neurite outgrowth, and formation of focal adhesions.

Plans:

Additional experiments will be conducted in a similar vein. We now have a need to establish procedures to make additional photoresist-coated cover slips either at here Michigan or back at the Cornell National Nanofabrication Facility.

3. Bioactivity of Protein Polymer Films *in vivo*

Progress and Plans:

The probes were coated with SLPL, SLPF, and SELP and were planted into Guinea Pig CNS. The animals were sacrificed and samples prepared for histological examination. Quantitiative histological examinations are underway.

We are currently developing plans to embed samples created only from the protein polymers themselves (as ~50 micron films or fibers) to isolate the influence of the polymer on the CNS, and remove the complication of the substrate. These experiments may be conducted with samples which were allowed to dry, or with their porous, phase separated structure remaining open by keeping them wet after processing and during surgery.

4. Outside communications

Christopher J. Buchko successfully completed writing his Ph.D. Dissertation on "Processing and Characterization of Protein Polymer Thin Films for Surface Modification of Neural Prosthetic Devices". Chris has since taken a permanent position with Guidant in San Francisco.

Shenkarram A. Athreya succesfully completed his M. S. degree on "Impedance Spectroscopy of Protein Polymer Modified Silicon Micromachined Probes", and has taken a permanent position with Applied Materials in Santa Clara, CA.

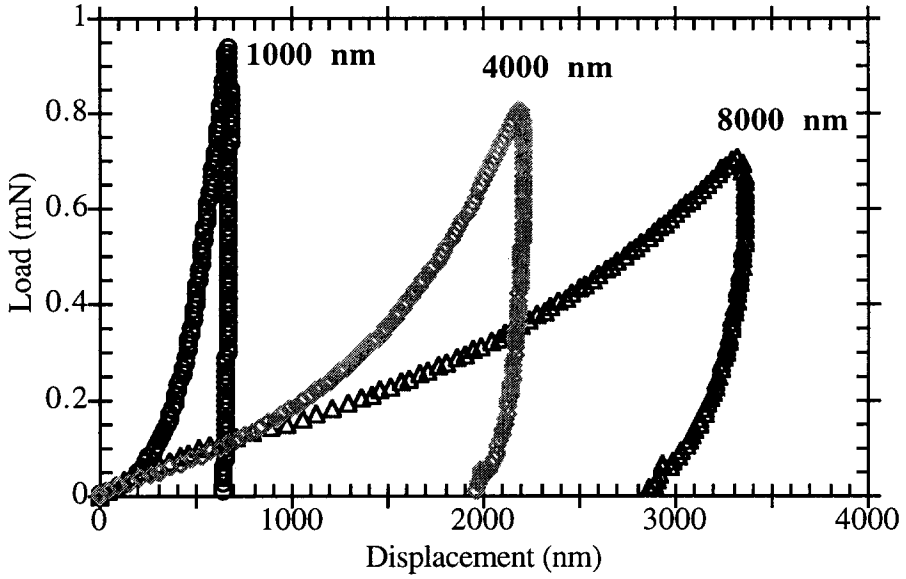
Micromachined probes for implanting into the toadfish were received from Dr. Allen Mensinger in the Department of Otolaryngology at Washington University Medical School. These were coated with SLPL / NGF fibers and were returned for implantation. Dr. Mensinger has reported that the coatings were also analyzed by them in their SEM and appeared adherent and filamentous. Initial results appear encouraging, and we anticipate coating an additional set for more testing.

A wire electrode probe array was also obtained from Daryl Kipke and Andy Schwartz and we are currently communicating with them to design a coating which would be suitable for their application. The assembly consists of a bundle of wires held in a 2-3 mm bundle, and should be suitable for either dip coating or electrospinning.

SEM of 100 mN Indented SLPF Thin Film

Figure 1.

Load-Displacement of SLPF Films of Different Thickness



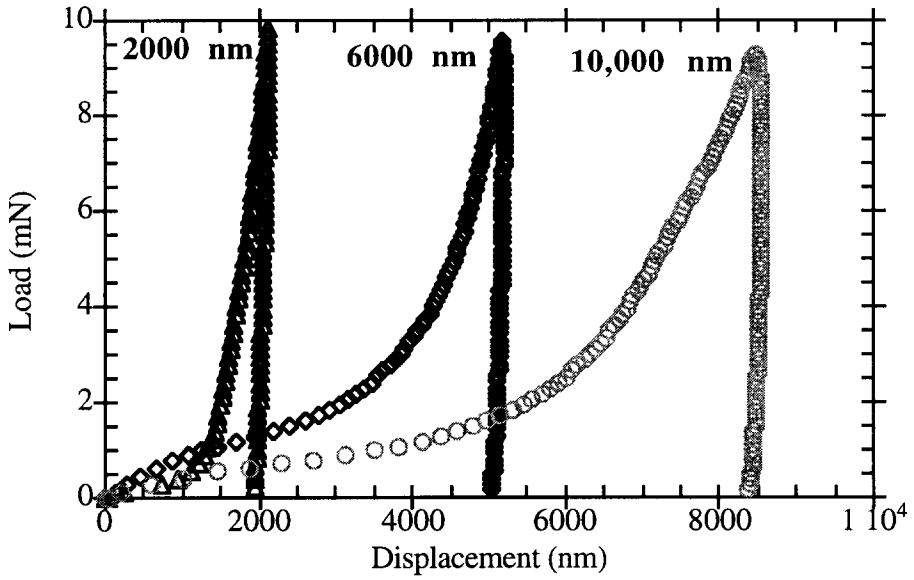


Figure 2.

Modulus Gradient in SLPF Thin Films

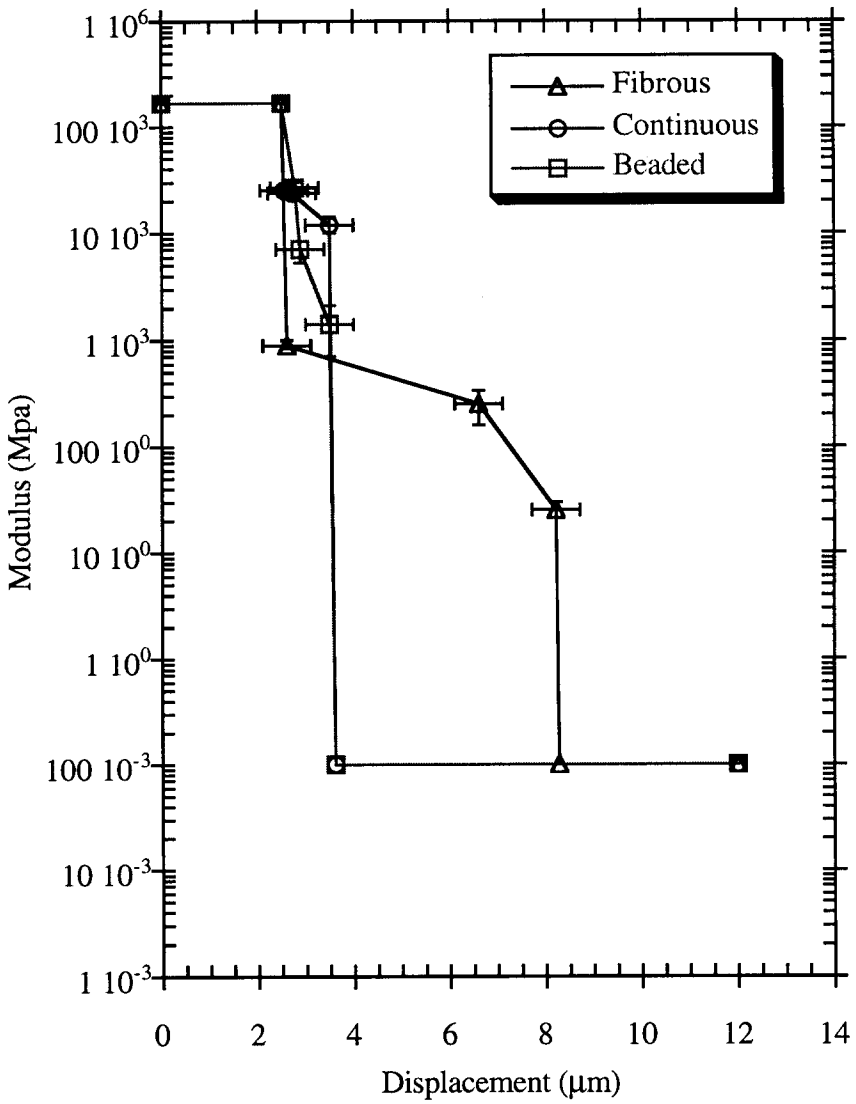


Figure 3.

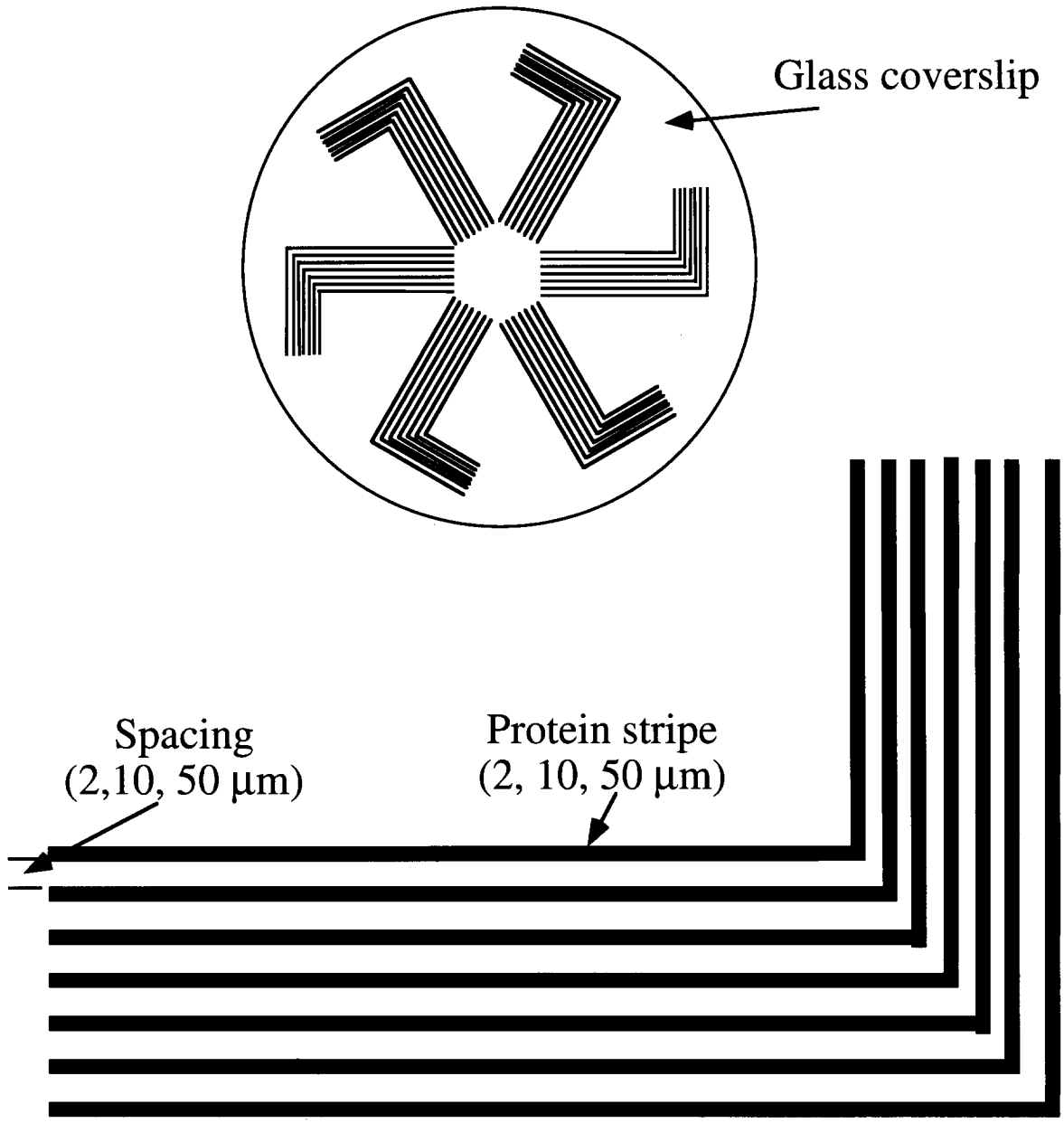


Figure 1. Design of patterned substrate.

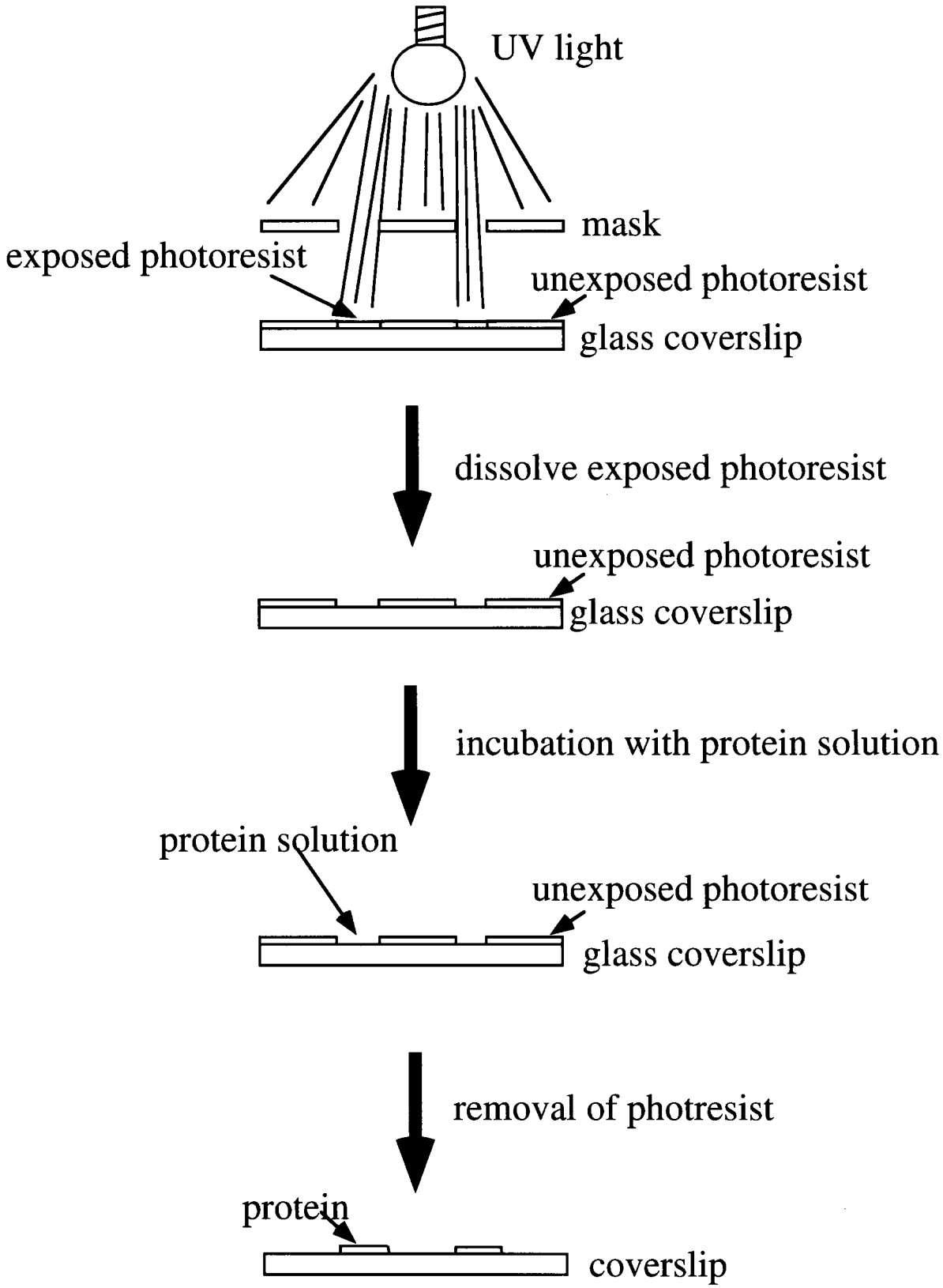
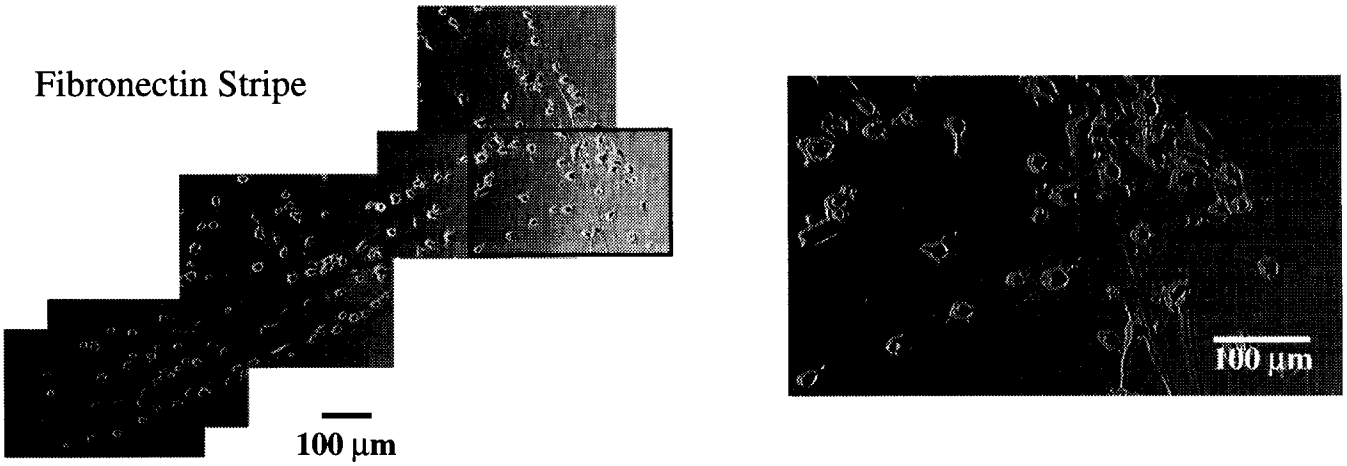


Figure 2. Schematic of protein patterning protocol



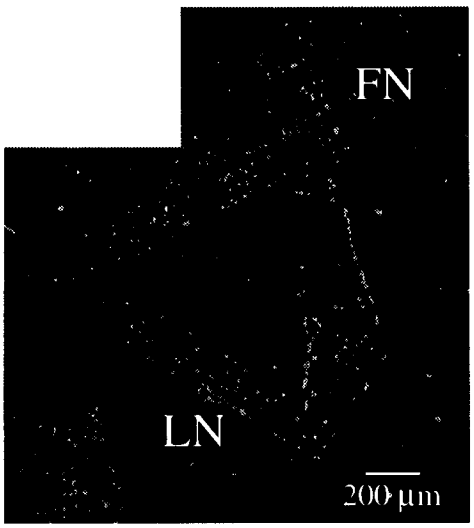


Figure 3. Patterned substrates seeded with Neuro-2A cells for 6 hours. Low magnification of coverslip patterned with alternating stripes of FN and LN (**middle**). Higher magnification of each type of stripe, FN (**upper**) and LN (**lower**) with insert of bend at dogleg.